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THE SYSTEMATIC AFFINITIES OF THE DIPTEROUS FAMILY PHORIDÆ.

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In a paper on the nomenclature of the dipterous wing published in the current number of *Psyche*, Professor Williston ('06) has again raised the much vexed question of the relationship of the Phoridæ among the families of Diptera.

During the past few years I have devoted considerable time to a study of this small and truly remarkable group of flies, but have been unable so far to reach any wholly satisfactory conclusion as to their proper systematic position. Attempts in a number of directions have each revealed some important if not insuperable obstacle, and I had let the matter rest, compelled to accept, albeit rather unwillingly, the final decision expressed by the late Baron Osten Sacken in the following words: "A real *affinity* with *Phora* does not exist anywhere."

In the nature of things such a negative opinion on a question of phylogeny can only be tentative, and it is with pleasure that I find the discussion reopened by so eminent a dipterist as Professor Williston.

Several entomologists who have given special attention to the group have attempted to reconcile its notable peculiarities of structure with types to be found elsewhere within the order of Diptera, and one has even essayed to connect them with the Aphaniptera which are almost unanimously regarded as forming a separate order.

The antennæ and the wings are the organs which offer the difficulties and I shall describe them briefly. The antenna consists apparently of a single large globular or pear-shaped joint

which bears a dorsal or terminal arista composed of three joints. When examined more closely, however, and more especially in longitudinal section, it is seen that this large segment is complex (see Fig. 1) and consists of a smaller joint almost entirely concealed within a larger outer one. These two are attached to the head by a third small and partially concealed segment. Such an arrangement is constant throughout the Phoridæ and is, so far as I know, absolutely unique among Diptera.

The nomenclature and homology of these joints has been interpreted in several ways. Wandolleck ('98) has considered the antenna to be six-jointed without attempting to homologize the segments with those of the antennæ of other Diptera. The majority of recent dipterists seem to have accepted a nomenclature which would regard the three large basal joints as homologous with the three segments of the typical brachycerous antenna, the apical one supplied with an arista of the usual kind. The third interpretation suggested in the paper before referred to by Williston ('06) is that the basal portion consists of two joints only, and that the arista so-called really represents the whole flagellum of the nemocera. Schiner also refers to the antenna as apparently two-jointed ('64, p. 335), but on page xv he says in more detail: "Fühler nahe am Mundrande eingefügt, scheinbar zwei-gliedrig, das dritte Glied rund (bei *Conicera* kegelförmig), mit nackter oder doch nur pubescenter Rücken- oder Apicalborste." It is thus evident that he takes them to be actually three-jointed, and in this view I fully concur. From the examination of large numbers of mounted specimens representing most of the described species, as well as several in microscopical section, and a number of published figures, I am fully convinced that there are always three distinct joints, the large apical one of which bears an arista.[1]

FIG. 1. Phorid Antenna. 1, 2, 3, antennal joints; *A*, arista.

[1] Whether we conceive the arista to be a modified portion of the nemocerous flagellum would in this connection be a problem apart from the relationship of the various brachycerous families among themselves.

In all species that I have examined the arista is three jointed, the two basal joints short and the other long. It thus offers no noticeable difference from the same appendage of many typical Brachycera.

Williston suggests that the Orphnephilidæ have antennæ comparable to those of the Phoridæ except for the number of aristal joints which is seven. In this case however, to judge from Schiner's description[1] the second joint is composite like the third joint of the Tabanidæ, Stratiomyidæ and allied forms. Whether the Orphnephilid antenna is in reality three-jointed I am unable to say, but the annulation of the second apical joint finds no counterpart among the Phoridæ.

Williston believes that the palpi are two-jointed, and bases a part of his argument for nemoceran affinities on this supposition. From my own observation I do not believe however, that such is the case, nor can I find any reference to this effect in the literature and published figures at hand,[2] with the exception of a single diagram by Dahl ('99, p. 75, Fig. 5) where he figures the palpi of the East Indian *Phora* (*Dorniphora*) *dorhni* with two joints. I think the accuracy of this one case can be questioned as I have identified almost certainly his species among a lot of Phoridæ from New Guinea and find the palpi single jointed as usual. Becker ('01) gives careful figures of the head with palpi in a number of genera[3] but in each case there is no indication of more than a single joint. I have studied with great care the mouthparts of several of the subapterous myrmecophilous genera, but have found no trace of a second joint. Fig. 2 is reproduced from a drawing of one of these species in which there is no trace of any articulation. The palpi of the Phoridæ are always large, and are in some instances immensely swollen (*e. g.*, *Phora palposa* Zett. and *Aphiochæta magnipalpis* Aldrich ♂) but in other respects

[1] In "Fauna Austriaca," II., 643, he says of the Orphnephilidæ: Fühler nahe am Mundrande stehend, kurz und so sich darstellend, als ob sie aus einem runden ersten, einem ovalen zweiten und einer Endborste bestünden; bei mikroscopischer Untersuchung zeigt sich, dass das zweite ovale Glied aus drei und die Endborste aus sieben walzenförmigen Gliedern besteht, deren letztes am Ende borstig ist.

[2] After this paper had gone to press, Dr. Williston called my attention to another published paper by Wesché (*Journ. R. Micr. Soc.*, 1904) in which Phorid palpi are figured as two-jointed.

[3] *Phora*, *Hypocera*, *Aphiochæta*, *Trineura* and *Metopina*.

they differ from those of the more specialized Brachycera like the Muscidea only in their more strongly developed bristles.

It is in the venation of the wings that the Phoridæ depart most strikingly from the other Diptera. This varies among the few known genera only in trivial details, so that the general type for the entire family is practically uniform. Fig. 3 shows the wing of a typical species. The veins can at once be divided into two groups, the several heavy ones which lie close together at the

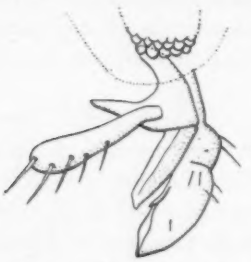

FIG. 2. *Commoptera solenopsidis* Brues, mouthparts of ♀.

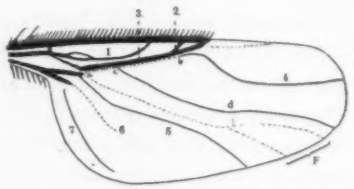

FIG. 3. Diagrammatic Phorid Wing. (After Becker.)

basal half of the wing anteriorly, and the four delicate ones which pursue an oblique course across the posterior part of the wing. This arrangement is so peculiar, that attempts to homologize the veins with those of other Diptera have proved very unsatisfactory. Several families present a somewhat similar appearance and in each case comparisons have been drawn. Williston, in the paper referred to above ('06) mentions the close similarity in venation to the Bibionid genus *Apistes*, and Becker ('01) earlier noted the resemblance to *Scatopse* another member of the Bibionidæ and to *Simulium* belonging to the Simuliidæ. It is true that there is a close superficial resemblance but no attempt has been made to homologize the veins. It is evident that *Apistes*, *Scatopse* and *Simulium* are all considerably specialized and we can indeed trace through the Bibionidæ the loss of the posterior series of veins which are quite strong in *Bibio*, weaker in *Dilophus* and still more so in *Scatopse* and *Apistes*, with a coincident strengthening of the anterior veins and their withdrawal basally. That a resemblance between this, the result of specialization within a family of closely similar forms and another otherwise widely different family

can have phylogenetic import, I am inclined to doubt. I think it can not be questioned that a complete venation is the primitive condition for the Bibionidæ, so that we cannot assume a common origin for the Bibionidæ and Phoridæ on evidence from neuration, but necessarily a *Scatopse*-like or *Apistes*-like ancestor for the Phoridæ. This is manifestly improbable as it involves after the fixation of a degenerating wing venation, vast strides in the evolution of other organs (antennæ, palpi) to give rise to Phoridæ while the parent stock remains unchanged.

Such a loss of posterior veins has occurred independently in many groups; the Stratiomyidæ and Hippoboscidæ may be mentioned among the Diptera and the Chalcididæ, Proctotrypidæ, Bethylidæ and Cynipidæ among Hymenoptera. The adaptation seems to be due to mechanical adjustment and of course not to common ancestry.

Palæontology offers but little on the history of the Bibionidæ and practically nothing on that of Phoridæ. The occurrence of *Phora* has been recognized by Berendt ('45) and Loew ('50) in Prussian amber. The bibionid genera *Protomyia*, *Bibio*, *Plecia*, and *Bibiopsis* have been found to occur together in considerable abundance in the Mayencian formation at Radoboj in Croatia (Heer, '47), while *Scatopse* is known first from the Ligurian, occurring in Prussian amber with *Phora* and also at Aix, France (Serres, '29).

In his monograph of the European Phoridæ, Becker has advanced the idea that the Phoridæ are derived from nemocerous forms allied to the Mycetophilidæ, basing his opinion principally upon a careful comparison of the wing venation of the two families made by Girschner. Girschner's view is given as follows: "Ich halte das Phora Geäder für ein modificiertes Mycetophilidengeäder, wie ich auch die Mycetophiliden für die nächsten Verwandten und Stammesangehörigen der Phoriden halte. Ich deute das Geäder in der oben skizzierten Weise (reproduced here as Fig. 3). Von *a* bis *b* ist die Discoidalader mit der Cubitalader verschmoltzen, wie dies schon angedeutet wird bei Macrocera. Bei einigen Phora-Formen kann man ziemlich deutlich — doch nicht so auffallend wie in der Zeichnung punctiert angegeben — die unter die Cubitalader hinstreichende Discoidalader wahrnehmen.

'Die kleine Querader fehlt, weil die in der Strecke *ac* enthalten ist."

This comparison is very striking and were it not for the fundamental differences in metamorphosis and in other organs, might be conclusive. It is further strengthened by the fact adduced by Becker that certain of the macrochætæ covering the body are in both families provided with chitinous bristle-like projections.

On the other hand, Schiner has with almost equal facility reduced the Phorid wing to a more generalized type of brachycerous venation according to the following scheme (see Fig. 4).

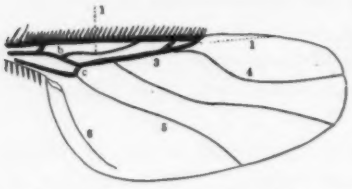

FIG. 4. Wing of *Phora* sp., as interpreted by Schiner.

The main differences between this and the arrangement by Girschner lie in the interpretation of the fourth vein involving the presence of the cross-vein and the presence of the anal vein. Schiner's designation of the indistinct thickening along the costa beyond the tip of the heavy veins as a "Fortsetzung der ersten Längsader" I cannot exactly understand, but it involves no important point.

Of the two views, that of Girschner accords more closely with the actual wing venation, and will I think be partly accepted with the exception of several points mentioned on a later page, by all who will study carefully a series of wings. That this similarity between the Mycetophilid and Phorid wing involves genetic relationship does not necessarily follow, however. As suggested on a previous page, specialization of wings often follows more or less parallel paths in diverse groups, and this seems to be especially true in those where a loss in complexity of structure is brought about by parasitism, secretive habits, or minute size. Turning to the Dipterous family Hippoboscidæ, widely removed from those previously discussed, we can see a strikingly similar condition to the one assumed by Girschner for the Phoridæ. Fig. 5 shows the wing of a species of *Olfersia*, a genus of Hippoboscidæ. The heavy veins are confined to the anterior basal region, while several oblique light veins tra-

verse the large posterior expanse. Moreover, the individual veins retain almost the same relative position in these representatives of the three families. In *Olfersia* the first, second and third longitudinal veins join the heavy costal vein, which extends for only a part of the wing length, in nearly parallel directions.

FIG. 5. *Olfersia* sp., wing. *ad*, adventitious vein; *4*, fourth vein.

The fourth is not fused with the third basally, but lies close to it so that the anterior cross-vein is extremely short. Such precisely similar developments are remarkable, and must, I think, be due to some similar tendency to degenerate in certain definite directions.[1]

Be this as it may, I think the evidence is sufficient to show that direct descent from Mycetophilid-like forms is not a logical necessity in tracing a genealogy which will account for the similar wing venation of the Phoridæ.

Several other attempts to discover relationships that have been less elaborately worked out are enumerated in the papers by Osten Sacken ('02) and Coquillett ('01). There is no agreement as to whether they belong properly to the Orthorrapha or Cyclorrapha. Osten Sacken (*l. c.*) refers them to his orthorraphous superfamily Energopoda which contains the Asilidæ, Empididæ, Lonchopteridæ and Phoridæ, while Coquillett has proposed the orthorraphous superfamily Phoroidea to contain the Lonchopteridæ and Phoridæ. Mik thought he saw Borborid affinities, as he says ('98, p. 205): "Aenliche Mundtheilen . . . findet man bei den Borboriden, mit welchen die Phoriden wahrscheinlich nahe verwandt sind."

The concensus of recent opinion associates the Lonchopteridæ and Phoridæ together with apparent good reason. The head, form of front with its macrochætæ, proboscis, palpi, antennæ with their arista, bristly thorax and legs, large, freely articulated ante-

[1] Such an idea savors of orthogenetic principles, although in an unusual sense, offering an interesting field for speculation, and for comparative studies as well.

rior coxæ, and the general habitus and actions when alive are very similar in both families. On the other hand the wing venation is different, but taken in connection with the facts already presented showing similar degeneration in so many families, this is not so great an objection as it would be otherwise.

Aside from Mik, dipterists do not appear to have detected many affinities with muscid forms. I am nevertheless inclined to agree with him that the Phoridæ show a relationship with the Borboridæ. This may quite probably also have been Schiner's idea when he placed the Phoridæ between the Bibionidæ and Borboridæ, rather than to show relationship to the former family as Williston suggests ('06). The various points of resemblance are: first, their similar appearance and actions when alive, they are the only family whose motions when running or flying could be confused with those of the Phoridæ; the modification of the incrassated hind metatarsi, the same segment being modified by rows of scaly bristles in every member of the Phoridæ, even the wingless forms and Termitoxenia; the tendency shown in the Borboridæ to lose certain of the apical and posterior parts of the veins in the wing and the tendency also to develop wingless forms like the Phoridæ. However, the legs and head of the Borboridæ are not bristly to any extent. Other differences are mainly the ones that appear equally in comparison with any family.

It is therefore in my opinion probable that the Lonchopteridæ and Phoridæ may together find a more suitable place in the Cyclorrapha rather than at the end of the Orthorrapha where placed by Coquillett. This is what Williston has done with them in the second edition of his manual ('96) where they are placed between the Platypezidæ and the Muscidea. At least there seem fewer objections to this course than to any other.

Regarding the nomenclature of the wing veins of the Phoridæ there has been considerable difference of opinion as mentioned on a previous page, and I would like to correct what I think is an error in the identification of the fourth and fifth veins. The first oblique light vein has been considered by various writers (Schiner, '62, Comstock, '94, Becker, '01, Brues, '04) as the fourth longitudinal vein, but I now believe that the second light

vein represents the fourth and that what has been called the fourth is an adventitious vein. The process of coalescence in the Hippoboscidæ has suggested this.[1] Here (see Fig. 6) the first light vein arises at the cross-vein which is near the middle of the third vein. This vein is undoubtedly the fourth on account of its association with the posterior end of the cross-vein yet it occupies exactly the same place as the second light vein in the Phoridæ. This is a far more probable sort of coalescence by shortening of the cross-vein than an approximation of veins throughout their entire length which would be necessary to bring the fourth vein near the tip of the the third where the adventitious vein originates. Other points in favor of an adventitious origin are the appearance in some cases of a nebulous thickening in the same space in the Hippoboscid wing and the great variability of this vein in the Phoridæ. It also removes the difficulty which Coquillett saw in recognizing three posterior veins in the Phoridæ which he thought would exclude them from membership in the Cyclorrapha. I think also that the slight thickening near the costa beyond the tip of the third vein is also an adventitious vein if it can properly be designated as a vein.[2]

I agree with Girschner and Becker that the sixth (anal) vein is lost, or obsolete, and that the last vein (fourth light vein) represents the seventh or axillary. There can be no doubt that the short anterior branch at the tip of the third vein represents the second which is fused with it to this point. In the more specialized genera (*e. g.*, Hypocera, Puliciphora) the fusion is complete to the tip.

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[1] There are many other developments among the Phoridæ which parallel those of the pupiparous Diptera such as the Hippoboscidæ. Briefly summarized they are (1) The degeneration and sometimes ultimate disappearance of the wings. (2) The production in the apterous or subapterous forms of very large eggs which distend the abdomen greatly and are even thought in some cases (cf. Wasmann,'02) to develop to the larval or pupal stage before being laid. (3) The similarity in wing venation already mentioned. (4) The degeneration of the eyes (cf. Streblidæ, Brues, 04[a], Fig. 1).

These resemblances are undoubtedly the result of parallel development, but as Professor Williston has suggested to me in a recent letter, they tend to weaken the value of the group Pupipara.

[2] Schiner refers to it as a part of the first vein.

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SOME OBSERVATIONS AND EXPERIMENTS ON THE NATURAL AND ARTIFICIAL INCUBATION OF THE EGG OF THE COMMON FOWL.

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Through a series of experiments on the developing chick, it was found that frequently the variations in development were so great that the value of the experiment was materially decreased. An attempt was therefore made to obtain more data regarding the following factors; position of eggs, turning of eggs, cooling of eggs, ventilation of eggs, moisture of eggs.

Before detailing the observations and experiments it should be emphasized that the eggs used were selected with much care. They were largely from the variety of fowls known as "Plymouth Rock." Especial care was taken to eliminate the effects of disease, inbreeding, poor food, etc. No eggs were taken from flocks in which there was not at least one cock for every ten hens. All mottled, rough shelled and ill-shaped eggs were discarded. Uniformity in size was next secured. The eggs were then tested by transmitted sunlight and those in the same stages of development selected.

POSITION OF EGGS.

That the position of the egg during incubation has an influence on the development of the chick was shown by Dareste ('91, p. 171). It is also a well known fact among poultry raisers that the position of the egg has a profound influence upon the growth of the chick. All seem to agree that if the small end of the egg is up, the head of the chick develops in this end, and as a result, many chicks will either be deformed or fail to free themselves from the shell at the time of hatching. With these points in mind, a series of observations was made with a view of determining just what position the eggs occupy during natural incubation.

Natural Position. — Two nests containing an aggregate of twenty-seven eggs were selected which were typical of the extreme conditions. One of these was exceedingly flat, being on the ground. The other, being in a box of straw, had a very concave bottom, so that the eggs were crowded closely together; those at the periphery always rested on a sloping side.

In each of these nests, the eggs were marked so that any change of position was readily noted. A diagram of the nest was made each day, showing the position of each egg and the angle of inclination of its long axis. The sketches were made at the time the hen was feeding. If she did not leave the nest, as sometimes occurred, she was gently removed until the sketch could be made.

The angles were assigned according to the following method: an egg whose long axis formed an angle of less than 10° with the horizontal plane was marked 0°. If the long axis formed an angle of more than 10° and less than 22½° it was recorded as 15°. If the angle were more than 22½° and less than 67½° it was recorded as 45 degrees. While those whose angles were more than 67½° were recorded as 90 degrees. It was found to be impossible to register the exact angle without the expenditure of much more time than the problem merited.

In the nest with the flat bottom there was an average of less than 10 per cent. of the eggs, in which the angle of inclination exceeded 15°. From this nest of twelve eggs eleven hatched, giving a percentage of 91.7.

In the second nest which was extremely concave an average of 30 per cent. of the eggs showed an inclination of 45° or more. From this nest containing fifteen eggs, thirteen hatched, giving a percentage of 86.6. Three other nests on the ground were compared with those in straw, but in these the percentage of chicks hatched was about the same. These observations led to the conclusion that the oblique position of the egg is a factor of little or no importance in natural incubation.

It should be added that in natural incubation, one very rarely finds eggs so placed that the smaller end is uppermost. This is probably the result of two mechanical factors. Those eggs at the margin, when beneath the hen, assume an oblique position

owing to the fact that this is the position in which least resistance is met; a second factor is that of specific gravity. The air space naturally tends to become uppermost and as this increases in size, the center of gravity becomes lower and lower, and the number of eggs assuming oblique positions increases as incubation progresses. It must, however, be kept in mind that an egg under natural conditions, does not maintain a position such that its angle is constant, and it may be that this varying angle of inclination influences development.

Experiments. — In order to ascertain what influence the position of the egg during artificial incubation has upon development, the following experiments were made:

In one tray of twelve-row capacity, the eggs were arranged in the following manner: Six rows were filled with eggs placed in such a position that their long axes were in a horizontal plane. Each row contained eight eggs, there being thus forty-eight eggs lying flat. In the remaining six rows the eggs were placed in such a position that their long axes coincided with an angle of 45°. Each of these rows contained ten eggs, giving sixty eggs placed obliquely. That the value of the results might not be lessened through the introduction of bad lots of eggs, they were controlled by taking equal numbers of these eggs from three different flocks of fowls and evenly distributing them throughout the tray.

On the fifth day thirteen infertile eggs were removed from those placed obliquely, leaving forty-seven living embryos. These eggs were again examined on the sixteenth day, and five dead embryos were found, leaving forty-two chicks alive at this time. Forty chicks hatched; the remaining two died after having pipped; the percentage of hatched chicks from the fertile eggs being 85.0.

From the forty-eight eggs lying flat, ten infertile eggs were removed on the fifth day, leaving thirty-eight live embryos. The sixteenth day, nine dead embryos were removed, leaving twenty-nine living chicks; of these eighteen hatched; three died after having pipped, and the remainder died in the shell. The percentage of chicks hatched from the fertile eggs was 47.3.

It is, of course, perfectly obvious that in this experiment, the

results indicated that eggs placed obliquely hatched a far higher percentage than those placed flat.

In a second experiment, the eggs were arranged in precisely the same manner, but in another incubator provided with a special ventilating apparatus. The fifth day, the eggs placed obliquely were tested and eight discarded. On the ninth day, three dead embryos were removed, and on the sixteenth day, four more were dead, leaving a total of fifty-three living eggs; forty-seven chicks hatched. Of the remaining six, four were dead in the shell, and two pipped. Thus there were hatched from those eggs placed obliquely, 88.7 per cent. of the fertile eggs.

From the forty-eight eggs placed flat, seven were found infertile on the fifth day. On the ninth day, six dead embryos were removed. On the sixteenth day, but one dead embryo was found. Thirty four chicks hatched. There was hatched about 83 per cent. of the fertile eggs.

The results of the foregoing observations and experiments taken together, lead to the deduction that when the supply of fresh air (oxygen) is inadequate, the oblique position of the egg, thereby bringing the embryo in closer contact with the air chamber, is decidedly advantageous. When there is an abundant supply of fresh air, there is but little to be gained through placing the eggs obliquely.

Turning of Eggs.

How many times the hen turns her eggs during the time of natural incubation is a question often asked, but as yet unanswered. Reaumer ('49, p. 166) states that they are turned daily and Dareste ('91, p. 161) that they are often turned twice daily. Believing this to be a point well worthy of investigation, a series of observations was made with the hope of obtaining some information as to the influence this factor plays in development.

Experiments have previously been made with a view of ascertaining what influence turning the egg has upon development. Dareste ('91, p. 165) placed sixteen eggs under the same conditions of artificial incubation. Eight were unmoved, while the remaining eight were turned twice each day. In the first set absorption of the yolk did not occur in any case; the embryos

died during the second or third week. Of the eight which were turned six developed normally; a seventh was opened on the twenty-second day, showing normal conditions; while in the eighth the chick died on the twentieth day, the adhesions between the allantois and the yolk having prevented the absorption of the latter.

Natural Turning. — In order to obtain satisfactory data, it was necessary to arrange nests in such a manner that the position of each egg could be sketched at frequent intervals without disturbing the hen. Three nests with felt sides and concave glass bottoms were constructed, and placed in such positions that the eggs could be viewed and sketched from below. The eggs were numbered in four places about equally distant from each other and midway between the two ends of the egg. Each figure bore an alphabetical index. The numbers on egg 1 would run thus: 1*a*, 1*b*, 1*c*, 1*d*, so that any degree of turning might be readily observed. Five sketches or plats of each nest were made each day during the incubating period, and at the following hours: 6 A. M., 9 A. M., 12 M., 3 P. M., 6 P. M.

The observations show that the eggs are turned partially or completely much more frequently than has been supposed, at least five times during any given day. It should also be stated that on a number of days sketches were made at more frequent intervals, and in nearly every case the eggs had been partially or completely rotated. The observations made were confined to the twelve hours between 6 A. M. and 6 P. M. A number of scattered observations lead to the belief that the eggs are also turned during the night, but just how frequently is yet to be determined.

The hen turns the eggs in two ways. If a sitting hen be watched as she returns from feeding to sitting, it will be seen that she moves her body rapidly from side to side. Whether the object be to turn the eggs is uncertain. Probably the first object is to bring the surface of the body in the closest possible contact with the growing embryos. Accidentally or purposely, she also turns the eggs. This is not only true of the hen returning from feeding, but also when on the nest, for she is frequently observed moving about and settling down with the same charac-

teristic lateral movements. Sometimes there are so many eggs in the nest or they are so widely scattered that the hen fails to properly cover them. When such conditions occur, the hen invariably uses her beak to bring the outlying eggs in contact with her body. Not only does she frequently thus turn the eggs, but also she very often reaches beneath her body and turns the eggs lying near the center of the nest. Why she does this is a question which awaits an answer.

Experiments. — Seventy-five eggs were selected from three lots of fowls, arranged in three groups and so placed that the eggs from one lot of fowls alternated with those from another lot. Those eggs of group I. were left unmoved; those of group II. were turned at 6 A. M., and 6 P. M.; those of group III. were turned at 6 A. M., 9 A. M., 12 M., 3 P. M. and 6 P. M.

From group I., five infertile eggs were removed on the fifth day, together with eight dead embryos, six of which had grown fast to the shell membrane. The eggs were again examined on the twelfth day and five more dead embryos were removed. These were examined and four were found to have the allantois grown fast to the yolk; but three chicks hatched from the entire twenty-five. The remaining four were dead in the shell. The number hatched was 15 per cent. of the fertile eggs.

In group II. there were three infertile eggs removed on the fifth day, and one dead embryo which had adhered to the shell membrane. On the twelfth day four dead embryos were removed, one had the allantois adhering to the yoke. In the others the cause of death could only be surmised. From the remainder, ten chicks were hatched; the others died in the shell, giving a hatch of 45.4 per cent. of the fertile eggs.

In group III. six infertile eggs were found on the fifth day and no dead embryos. On the twelfth day one dead embryo was found. Eleven chicks hatched; two died after having pipped, while the remaining five were dead in the shell; the number of hatched chicks being 58 per cent. of the fertile eggs. The experiments indicate that frequent turnings (at least five) give best results.

It is necessary to point out, however, that a very low percentage of chicks hatched even in group III. This is to be attributed

directly to a lack of sufficient oxygen, the incubator in which the experiments were made being poorly ventilated, owing to the fact that the ventilating system had been modified in order to compare its results with those provided with special ventilation. A second incubator provided with a special ventilating apparatus, hatched 83 per cent. and 88.6 per cent. of two lots of eggs taken from the same fowls.

Dareste concluded from his experiments that during the first week of artificial incubation, eggs which are unturned develop in essentially the same manner as those which are turned. The principal cause of death is due to the allantois growing fast to the yolk, causing the rupture of the vitelline membrane, thereby allowing the yolk to escape so that it cannot be taken into the body of the embryo. Dareste adds that when the eggs are turned it is probable that the position of the allantois is shifted, and this movement prevents its adhesion to the yolk. It should be remarked that during the early days of incubation it is also necessary to turn the eggs frequently; otherwise, the embryo grows fast to the shell membrane. This has not only been shown by the preceding experiments, but has been repeatedly observed in other eggs.

IV. Temperature of Eggs.

Repeated attempts have been made to ascertain the temperature of the egg during natural incubation, but as yet the results are far from satisfactory. This is due to the difficulty experienced in testing the temperature of different parts of the egg. The fact that the egg comes in contact with a heating surface above and a cooling surface below, leads to most perplexing complications. While the temperature of the hen is easily ascertained, it is not an easy matter to know the precise degree of heat applied to the surface of the egg. Moreover, it should be kept in mind that during incubation, not all the eggs are at all times in contact with the body of the hen. A layer of feathers intervenes to modify the temperature, this layer varying greatly in thickness in different parts of the body, and at different times during incubation. All these factors conspire to make an exceedingly difficult problem.

Natural Temperature of Hen. — In attempting to determine the daily temperature of the hen, special self-registering thermometers were fastened to blocks so cut that their upper surfaces were nearly egg-shaped. The lower surfaces of the blocks were broad and flat, so that they could not be easily overturned. One was placed in each of four nests and left for two or three hours, when the reading was made.

The following table shows the temperatures obtained by this method, during twenty days of incubation. In this as in subsequent tables, the Roman numerals indicate the serial numbers by which the hens were designated, while those above the columns indicate the day of incubation.

	1	2	3	4	5	6	7	8	9	10
I.	102.1	103.0	103.0	103.8	105.0	104.5	105.0	105.0	106.2	106.0
II.	103.0	104.0	103.5	104.5	104.5	104.0	105.0	105.5	104.5	104.6
III.	102.0	102.0	103.0	103.0	105.0	105.0	104.5	104.0	104.5	104.0
IV.	101.5	102.5	102.5	103.0	103.5	104.0	104.5	104.5	105.0	105.0
	11	12	13	14	15	16	17	18	19	20
I.	105.0	104.5	105.0	105.5	104.5	105.5	104.8	105.0	104.5	105.5
II.	104.6	104.5	104.6	104.2	105.0	104.8	105.0	105.0	105.0	104.0
III.	104.0	105.0	104.0	103.6	104.0	105.2	104.2	103.5	103.0	104.0
IV.	104.8	105.0	104.5	105.0	104.8	105.0	105.0	104.5	104.5	105.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course, somewhat unsatisfactory, since the excitement of the fowls due to their being removed from the nest, results in a temperature somewhat higher than the normal.

	1	2	3	4	5	6	7	8	9	10
I.	103.0	104.0	103.5	104.6	105.5	105.0	106.0	106.0	105.5	106.0
II.	104.0	105.2	105.5	105.5	105.5	106.5	105.2	106.0	106.0	107.0
III.	103.5	103.2	105.5	106.5	106.2	105.0	105.2	105.0	105.0	105.0
IV.	102.0	102.5	104.0	104.5	105.0	105.0	104.5	105.0	105.0	105.5
	11	12	13	14	15	16	17	18	19	20
I.	106.5	105.8	105.6	105.5	106.2	106.2	106.0	105.5	105.8	105.5
II.	106.2	106.5	106.5	106.0	107.4	106.0	106.0	106.5	106.2	106.5
III.	105.8	105.0	105.2	106.2	106.5	106.0	106.5	106.0	106.2	106.0
IV.	105.0	105.2	105.6	106.0	105.5	105.2	105.0	105.0	104.8	105.0

Natural Temperature of Egg. — Since experiments show that

the above temperatures are too high for artificial incubation, it is necessary to push the inquiry a step further with a view of determining the exact temperature of the egg during natural incubation. The temperature of the hen recorded below was obtained from a thermometer attached to a block as described above. The temperature of the egg was taken in the following manner: A pail of lukewarm water was brought to a temperature of 98° F. (by the addition of warm or cold water). The egg was then placed in a tightly fitted rubber bag and held about four inches below the surface of the water. An opening was then made in the shell directly over the embryo, and a self registering thermometer warmed to 98° inserted for five minutes. The thermometer was inserted just far enough to bring its lower end at the center of the egg. As often as the eggs were broken for testing, they were replaced by eggs taken from other hens set at the same time.

	1	2	3	4	5	6	7	8	9	10
Hen.	102.2	103.0	103.5	104.0	103.8	105.0	104.6	104.5	105.0	105.0
Egg.	98.0	100.2	100.5	100.5	100.4	101.0	101.8	102.5	101.6	102.0
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.2	104.5	105.0	105.2	105.0	104.6	104.8	104.5	104.5
Egg.	101.8	102.2	102.0	102.5	102.0	103.0	102.4	103.0	103.0	103.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course somewhat unsatisfactory since again the excitement of the fowls, due to their being removed from the nests, resulted in temperatures somewhat higher than normal.

	1	2	3	4	5	6	7	8	9	10
Hen.	103.0	105.0	104.8	104.2	105.2	105.0	104.8	104.8	105.0	105.0
Egg.	99.5	100.0	100.2	100.5	100.6	101.0	100.5	100.5	101.5	101.5
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.0	104.8	104.8	105.2	105.0	105.5	104.0	104.0	104.0
Egg.	101.5	101 2	100.8	101.8	102.0	101.8	102.2	102.0	102.4	102.4

While the above are the only sets of daily observations, they were supplemented by a number of scattered tests. In no case

was the egg found to exceed the temperature given in the table by more than one degree. In but few cases was it found to be a degree lower. Although it cannot positively be stated that these tested eggs would have hatched, the inference seems more than probable, since in five other cases where hens were set on eggs from the same flocks of fowls, the fertile eggs hatched with but very few exceptions. These observations show that the proper incubating temperature of the egg is about 100° for the first week; 101° for the second, and 102°–103° for the final week.

Temperature of Artificially Incubated Eggs. — The next problem is to determine what temperature must be kept in the air chamber of the incubator in order to obtain the above temperature of the egg. A series of observations was made on the artificially incubated egg. The temperature of the egg chamber was read from a thermometer placed flat and on a level with the top of the eggs, but not in contact with them. The temperatures of the eggs were taken in precisely the same manner as in the preceding experiment.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	103.0	103.5	103.0	104.0	103.5	104.0	103.0	103.5	105.0	103.0
Egg.	100.2	100.0	100.0	101.2	101.6	101.8	101.6	100.0	102.8	101.0
	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	104.0	105.0	103.0	104.5	103.0	105.0	104.5	106.0	106.0
Egg.	102.0	102.6	103.7	102.5	103.6	104.8	104.0	104.0	—	—

The above record was made from an incubator which hatched about 85 per cent. of the fertile eggs. The hatch, however, was somewhat premature, since many of the eggs hatched on the nineteenth day. It is thus evident that a temperature somewhat too high had been carried. In view of the irregularities of the incubator a second experiment was made.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	102.0	102.0	103.0	102.0	102.5	103.0	102.5	102.0	103.0	103.5
Egg.	99.5	100.0	101.0	100.5	100.5	101.0	100.0	100.0	101.0	101.5
	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	103.5	104.0	103.5	104.0	104.5	104.0	103.5	104.0	104.5
Egg.	101.5	101.8	102.0	102.5	103.0	103.0	103.0	102.5	102.5	103.5

As will be observed, the incubator was under better control in the second experiment and the chicks hatched on the twentieth and twenty-first days yet the percentage hatched was about the same as in the first, not including in either case the eggs destroyed in making the tests. While a more extended series of tests would be highly desirable, one certainly does not widely err in stating that the most favorable temperature within the egg chamber is close to 102°–103° F. the first half of the incubating period and 103°–104° F. for the latter half.

In any consideration of temperature, the fact must be kept in mind that as the chick grows, it gives off more and more heat, so that if an incubator of 200 egg capacity were entirely without artificial heating, the temperature would be much higher than that of the surrounding atmosphere; it consequently follows that less artificial heat is necessary during the later stages of incubation. The 102°–103° in the earlier stages is largely artificial heat, while the 103°–104° in the later stages would be the combined animal heat, given off by the egg, and the artificial heat supplied by the heat radiator.

V. Cooling of Eggs.

How frequently the eggs should be cooled and for how long a period, is a question of considerable importance. Not being entirely satisfied with the data at hand, an attempt was made to gather some information by actually watching a number of hens from day to day.

Natural Exposure. — Six hens were observed throughout the period of incubation and the results tabulated. It was found that the average time the hen leaves her nest, during the first fifteen or eighteen days is about thirty minutes. During the last few days of the incubating period she rarely leaves the nest. The longest time a nest was left exposed was an hour and twenty minutes, and the shortest time about twelve minutes. If the hen be obliged to forage for food, she remains a much longer time than when food is at hand. But one or two instances were noted in which the eggs were exposed for much more than an hour. It may be stated with a fair degree of certainty that the cooling of the eggs is due to the necessity of obtaining food, and in no way funda-

mentally affects the growth of the chick when there is an abundant supply of fresh air. There is not the least doubt, however, but what it has a beneficial influence in cases of poor ventilation, and since no incubator is supplied with too much, it probably is best to adopt the common practice of cooling the eggs. In so doing it would not seem advisable to cool the eggs for more than twenty to thirty minutes each day, for the first fifteen or eighteen days.

VI. Ventilation of Eggs.

Natural Ventilation. — In natural incubation a perfect ventilation exists. An abundance of fresh air can always reach the eggs by diffusing through the feathers which cover them. This process is constantly going on during incubation, the foul air likewise having free exit. There is thus ample opportunity for a continuous circulation of air, and there is every reason to believe that it takes place. There is also afforded by the feathers a complete barrier against sudden draughts of air. The fresh air is also raised to a certain temperature through the heat of the hen before it comes in contact with the eggs, which also serves to reduce any excessive humidity. This perfect system of ventilation cannot fail to impress one of its importance in facilitating the growth of the chick.

Dareste ('91, p. 150) conducted the following series of experiments: All the apertures of the incubator were closed during incubation, and upon examination it was found that nearly all the embryos had died. It was found further, that there had developed in the albumen a microscopic organism resembling the ordinary yeast plant. The author concludes that air modified by embryonic respiration, facilitates the growth of parasitic organisms.

Gerlach ('82, p. 115) found that by diminishing the quantity of air during incubation, he could cause dwarfing of the embryo. He then tried whether an increase in the size of the embryo could be brought about by increasing the quantity of air. A part of the shell was scraped very thin and placed in an incubator. During the first two days the normal and modified eggs were alike, but after that time the embryo in the scraped eggs developed at a remarkably rapid rate, nearly twice as fast as in normal growth.

A second method of increasing the supply of air was to remove whole pieces of the shell. Of course great care was taken not to injure the shell membrane or growing blastoderm. This fracture was made some distance from the embryo, so that the drying could not extend to the embryo, and the egg after the removal of the part of the shell was turned so that the broken portion was downward. The embryo was perfectly formed, but grew at the same astonishingly rapid rate.

The above consideration led to the conviction that artificial incubation can only proceed where there is an abundant supply of fresh air (oxygen). In order to confirm this supposition, the following experiment was tried.

Experiments. — Two incubators with similar ventilating systems, which, however, were believed to be inadequate, were employed. One was left with the ventilating system unmodified. The other was provided with two one-inch intake pipes. These extended to the outside of the building in which the incubators were located, and so arranged that a continuous current of fresh air passed into the egg-chamber.

Two egg trays of 100 capacity each, were filled with eggs from the same lots of fowls; special care being taken to divide the eggs from each flock so that there should be an equal number in each tray. The eggs were then subjected to exactly the same treatment, barring slight variations in temperature which necessarily existed.

On the fifth day the eggs were tested, and from the incubator with special ventilation, sixteen (infertile) eggs were removed. From the other, twelve (infertile) eggs were removed. On the twelfth day they were again examined. From the incubator with special ventilation seven dead embryos were removed and from the other, twenty. From the eighty-four in the incubator with special ventilation, seventy-two hatched, while five were dead in the shell, giving a percentage of 85.7 per cent. hatched from the fertile eggs. Of the eighty-eight eggs remaining in the other incubator, but thirty-nine hatched; a number of the remainder pipped, just how many was not recorded, while a large number were dead in the shell. There was thus hatched in the incubator without ventilation 44.3 per cent. of the fertile eggs.

But when a perfect ventilation has been obtained, it has produced certain deleterious effects which must be corrected. It is commonplace to say that when evaportion goes on in still air, this air soon becomes saturated, and evaporation, if not stopped, goes on very slowly. If, however, the saturated air is constantly removed and dry air takes its place, the rate of evaporation is increased. It is thus evident that any discussion of ventilation must take into consideration the question of moisture.

VII. Moisture of Eggs.

There is probably no one factor so little understood as that of moisture. The most careful observations of the nesting habits of the hen seem to only complicate the matter. A hen may build her nest on the ground, or in the hay loft, and in each case hatch about the same percentage. These facts, which are a matter of every-day observation, lead us to believe that eggs hatch equally well under these variable conditions. The moisture necessary for development must then be controlled by the hen, or egg, or both.

There are certain constant factors in the production of moisture which we may accept as existing. First of all, the temperature of the air in the nest is far higher than that of the outside air. As the two come in contact, there is more or less moisture produced. A second source is from the perspiration of the skin. A third source is from the egg itself. These three sources supply, so far as we are able to determine, the moisture necessary for the normal development of the egg.

It is known through the experiment of Reaumer, that excessive moisture gives rise to the pathological forms. Dareste (p. 159) also records an experiment in which the atmosphere was saturated and as a result the albumen liquified and leaked through the shell. Furthermore, Dareste, stated that excessive moisture facilitates the growth of parasitic forms which develop in the albumen.

The writer made a series of daily hygrometer tests with a view of ascertaining just how much moisture existed in the nests. In testing with the hygrometer, it was placed in the nest among the eggs, and at the end of fifteen minutes was taken out

and the reading recorded. Although a great number of these tests were made and tabulated they were later discarded, owing to wide variations in the hygrometers and the problem attacked in another way. It is of course well known that the egg decreases in weight during incubation and that this is due chiefly to the evaporation. In order to find out definitely how much evaporation goes on during natural incubation, thirty-six eggs were weighed each day for twenty days and these weighings tabulated. It was found on the average that the egg during natural incubation loses about 13 per cent. of its original weight.

It was also found by experiment that the evaporation could be lessened until the egg lost but 9 per cent of its original weight and still give a healthy chick. It was likewise learned that evaporation could be increased up to about 20 per cent and the eggs give rise to perfect chicks. It would thus appear that the moisture in the incubator should be so controlled that it will allow the evaporation of about 13 per cent of the original weight of the egg.

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A PECULIAR PELVIC ATTACHMENT IN NECTURUS MACULATUS.

CLARA HEPBURN.

While studying the vertebral column of *Necturus maculatus*, in the regular class work, I found in my specimens a peculiar form of pelvic attachment which seems remarkable enough to mention.

The pelvis was attached to the eighteenth vertebra and, in addition, there were two rudimentary ribs on the nineteenth. There was nothing strange about the twentieth but on the twenty-first there was one half of a hæmal spine, that of the right side, the first complete hæmal arch being on the twenty-second.

For comparison in order to show its anomalous character, it is necessary to give the statistics concerning the conditions as reported hitherto. F. Smith, '00, and Wilder, '02, have summarized the results hitherto reported, including the papers of Parker, '96, Bumpus, '97, and Waite, '97.

In one hundred and fourteen specimens tabulated by Smith, the sacral vertebra was the nineteenth in eighty-one cases, the twentieth in sixteen and the twenty-first in one, this latter being the most posterior position ever recorded. In twelve cases the pelvis was oblique and of these three made use of the eighteenth on one side. In four cases there were three sacral ribs, that is, two upon one side and in one of these, figured by Smith on page 638, the ribs involved were the eighteenth on the right and the eighteenth and the nineteenth on the left. Thus in Smith's one hundred and fourteen cases, four involved the eighteenth vertebra but all were asymmetrical. The one which approached nearest my specimen is the one figured by Smith, where there was a ligamentous connection between the rib rudiment on the nineteenth and the sacrum, and as my specimen had been macerated before I examined it, I cannot tell whether there was such a connection or not.

As Wilder has used the same authors for his summary as has Smith, it is unnecessary to give his report.

None of these authors mention a case with the symmetrical pelvic attachment of both sides upon the eighteenth vertebra, nor one with symmetrical rudimentary ribs on any vertebra posterior to the sacral connection. It is rather significant that the nineteenth vertebra, the one that in the majority of cases bears the sacral ribs, should, in my specimen, have borne the rudimentary ones.

FIG. 1.

The fact that in this particular case, the first entire hæmal arch was found on the twenty-second vertebra, is not so remarkable. F. Smith records this position in sixty-two cases out of two hundred and forty-one. Wilder, also, states that the hæmal arch appears suddenly on the twenty-second to the twenty-fourth vertebra, usually the twenty-third. He then adds that in one case the vertebra just anterior to the first one bearing a complete hæmal arch, bore upon one side a slender process, four to five millimeters long, evidently representing an incomplete hæmal arch. But whether the vertebra which bore the complete hæmal arch was the twenty-second, twenty-third or twenty-fourth he does not say, so that one cannot tell whether the vertebra with the incomplete hæmal arch was the twenty-first, as in my specimen, or one of the two next posterior.

Although Bumpus has shown by a careful comparison that the variation of the position of the first hæmal arch is entirely independent of that of the sacral vertebra yet the presence of even a partial arch upon a vertebra anterior to any previously recorded, when taken in connection with the extreme anterior location of the sacral connection, appears important.

Summarizing, then, the points of especial interest in the case in question, we have the following:

1. The pelvic attachment to the eighteenth vertebra on both sides.

2. The presence of a pair of rudimentary ribs upon the nineteenth vertebra, the position of the usual pelvic attachment.

3. The presence of an incomplete hæmal arch upon the twenty-first vertebra.

SMITH COLLEGE,
December 11, 1906.

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NOTE ON THE CONUS ARTERIOSUS OF MEGALOPS CYPRINOIDES (BROUSSONET).

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Since describing the conus arteriosus in *Tarpon Atlanticus*[1] I have been fortunate in securing a specimen of *Magalops cyprinoides*. For this I take the present opportunity of thanking Professor David Starr Jordan.

The fish in question, preserved in alcohol, measures 19 cm. (including caudal fin) so that the heart is extremely small, and is, on account of its somewhat friable condition, difficult to handle.

The conus is everywhere quite obvious from the exterior. Fig. 1, drawn from the left side, indicates that the general form of

FIG. 1.

FIG. 2.

FIG. 1. Entire heart of *Megalops cyprinoides* from the left side, × 3. A small portion of the atrium has been removed to display the conus more fully.

FIG. 2. Frontal section through the right side of the heart of *Megalops cyprinoides*, showing the relations in the conus region, × 10.

conus and bulbus resembles that of *Amia* rather than that of *Tarpon*.

The heart was opened by a mid-ventral sagittal incision, it having been previously ascertained that such an incision would fall between the valves without cutting them. After examination and

[1] BIOL. BULL., February, 1907, p. 145. (The literature on the conus arteriosus is given in this article.)

measurement the two halves were imbedded in celloidin and cut into serial sections.

The extreme length of conus is 1 mm. in the mid-ventral line and 1.5 mm. in the mid-dorsal and lateral lines. The ventricle, from apex to junction with conus, has a mean measurement of 5.5 mm. The proportion in mean length of conus to ventricle is therefore 1 to 4.

The conus contains two transverse tiers of valves, each tier consisting of a right and left cusp placed symmetrically with regard to the mid-sagittal plane. The general arrangement agrees closely with that found in *Tarpon*, but the proximal cusps appear to be proportionately more capacious.

The conus in *Megalops* not only projects more freely from the ventricle than in *Tarpon*, but is of greater proportionate length. It would seem to resemble more closely the conus of *Albula* (as described by Boas, '80) except in the absence of the subsidiary valve cusps of the latter.

It should be noticed that the heart described is from a young fish, also that the measurements are, at best, approximate; therefore, comparisons with adults of other genera, if pushed too closely, are apt to be misleading.

The atrio-ventricular valve has three cusps.

A specimen of *Chanos chanos* (Forskål), for which I also have to thank Professor Jordan, presents an easily recognizable vestigial conus arteriosus, but only one tier of valves.

ST. LOUIS, MO., February 1, 1907.

SEX DIFFERENTIATION IN LARVAL INSECTS.

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The question of the causes of sex differentiation is a problem to the solution of which we seem now to be only a little nearer, despite numerous researches, than we were many years ago. It is advisable, perhaps, to continue to attempt to overcome some of the outworks of this well entrenched problem. One of the outliers of the main problem may be described in the phrase "When is sex differentiated?", another in the phrase "Does nutrition affect sex?"

Being engaged in rearing experimentally large numbers of silkworm moths, *Bombyx mori*, I have taken advantage of the

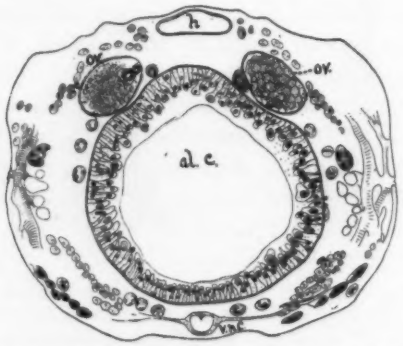

FIG. 1. Section through female *Bombyx* larva, just after the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord.

opportunity to test for this species both these subsidiary parts of the sex differentiation problem. Various lots of larvæ were set apart, each individual being isolated so as to insure identity of nutrition conditions, and fed on short rations. The result of these experiments is given in a paper in the *Journal of Experimental Zoology* (vol. 1, pp. 357–360, 1904). It is sufficient

to say here, in a word, that this "short feeding" produced no apparent effect in determining the sex of the moths.

Since these experiments I have learned a good reason why

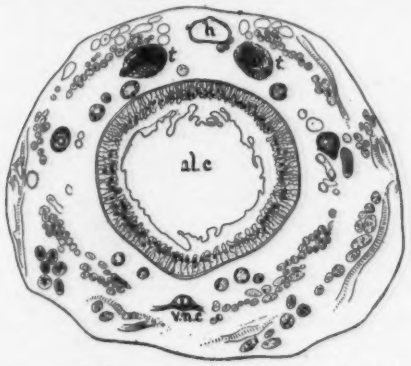

FIG. 2. Section through male *Bombyx* larva, just before the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

the nutrition of the larva, at least after its first moulting, should have no effect on the sex as revealed in the moth, and this reason is that the sex of each individual is definitively determined at

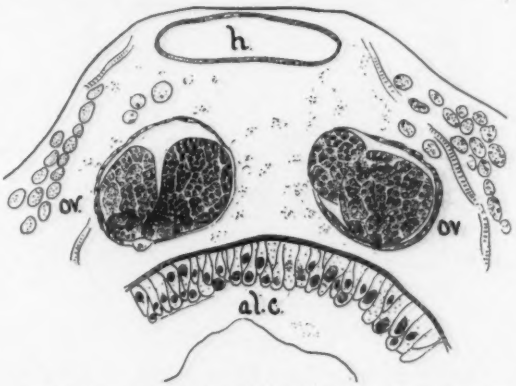

FIG. 3. Section (dorsal third) through female *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

least as early as immediately after the first larval moulting, as may be readily perceived by an examination of the rudimentary reproductive glands.

In the larval silkworm there may be noted on the dorsal wall of the fifth abdominal segment two low tubercles, rather dark colored. Directly beneath these spots lie the developing reproductive organs (ovaries, testes). By dissecting a number of

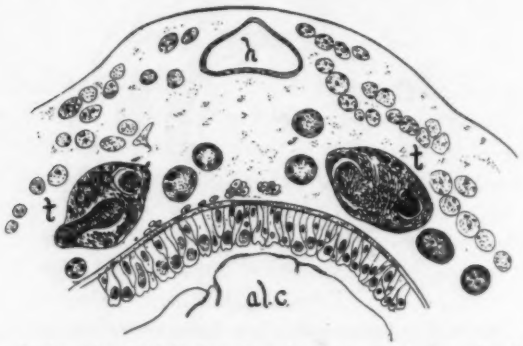

FIG. 4. Section (dorsal third) through male *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

larvæ in their last or next to last intermoulting period, a marked difference will be noted in the size of the organs in different individuals. By dissecting out the organs and sectioning them,

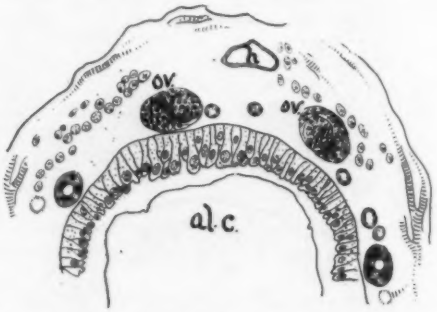

FIG. 5. Section (dorsal half) through female *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

constant marked histologic differences will be seen in the two sizes of organs. As a matter of fact the larger are ovaries, with well-developed rudiments of egg-tubes, the larvæ possessing

them being female individuals, while the smaller are testes. By dissecting larvæ of successively younger age, these differences in size and histologic character may be followed back to just after the first moulting.

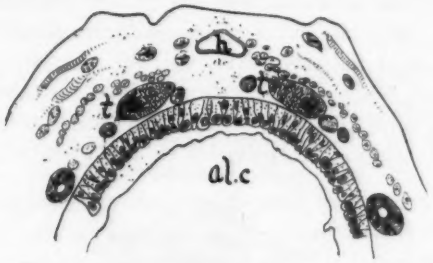

FIG. 6. Section (dorsal half) through male *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

Figs. 1 to 8 show, with little need of explanatory description, the gross character of the histologic differences, the relative sizes (in cross-section) of the two kinds of organs, and also the rela-

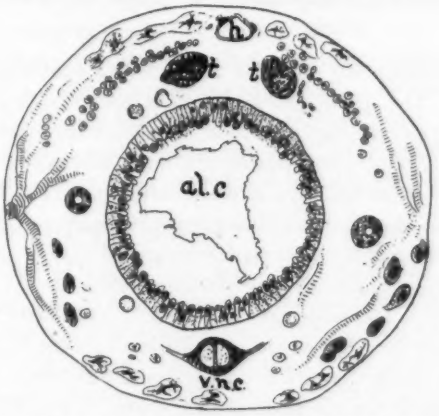

FIG. 7. Section through male *Bombyx* larva, just before the first moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

tive size of these organs in the various larval stages. I have not been sure of being able to distinguish between the sizes or the histologic characters of the reproductive organs in the just

hatched larvæ, but even here the organs are conspicuous and well started in development, being larger in diameter than the heart or the spinning glands.

We may affirm then positively that from the time of the first moulting on the silkworm larva has its sex determined: its reproductive glands are ovaries or testes distinguishable by obvious histologic characteristics. Also that the just hatched larva has reproductive organs already well developed. I have little doubt that careful scrutiny of the organs at this stage would reveal to the trained histologist, especially to the student of oögenesis

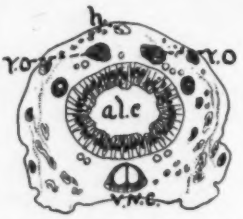

FIG. 8. Section through *Bombyx* larva (male or female?), two days after hatching; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *r*, *o*, reproductive organs.

and spermatogenesis, definitive characteristics marking sex differentiation. The sex of the silkworm is not to be tampered with by gorging or starving, and what is true of this lepidopteron is undoubtedly true of its cousins, the other moths and the butterflies. It is probably also true of other insects with complete metamorphosis. I recall dissections of various larvæ, notably of *Corydalis cornuta* (a neuropteron) and of *Holorusia rubiginosa* (a dipteron) in which the reproductive organs appear of two sizes in specimens of the same age: indeed in *Corydalis*, of two shapes. These organs need histologic examination. Some student should laboriously work through a long and representative series of insects and settle the question as to the time of sex differentiation. That is, find out whether it be true for all, as it is in the silkworm, that the time of sex differentiation is obvious before, or, at latest, at very little after the time of hatching. If it is true, the question of the influence of nutrition in sex determination will also be settled — for insects. And we need waste no more time in tedious feeding and tabulating.

FURTHER NOTES ON THE REGENERATION OF THE FINS OF FUNDULUS HETEROCLITUS.

G. G. SCOTT.

During the last summer the writer while engaged as assistant at the biological laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass., conducted various experiments on *Fundulus heteroclitus*. Since a number of these can be conveniently grouped under the heading *regeneration* they are collected and presented in the present paper. Various factors determining regeneration have been under discussion and it was thought that these should be tested using *Fundulus h.* as a type since it is a representative teleost and that therefore results might differ from those obtained in crustaceans, for example, and that certain principles deduced from crustacean work might possibly have to be modified. Thanks are due to Dr. F. B. Sumner as director of the laboratory of the Bureau of Fisheries for assistance in providing the necessary apparatus and other facilities.

The following experiments were designed to show: (*a*) the relation of temperature to regeneration, (*b*) the relation of degree of injury to regeneration, (*c*) the relation of length and weight (*i. e.*, age) of specimen to regeneration, (*d*) the relation of amount of food to regeneration, (*e*) adaptation in regeneration.

(A) Relation of Temperature to Regeneration.

With a view of testing this the caudal fin of ten *Fundulus h.* were cut in a line as near as possible to the origin of the caudal fin rays. The same procedure was followed in all the later cases to be described. These fish were placed in a chest or jar with three liters of sea water and the jar placed in an ice chest where the temperature averaged 14°C. The water was changed every day or so. On August 24 five fish had survived. The fish had not been fed. The survivors had been under observations for 41 days or almost six weeks. But in those survivors there was no sign of regeneration. With fishes in normal conditions regenera-

tion is evident after the first few days after operation. In order to further test this point and ascertain whether lack of food had anything to do with the result, two lots of fishes were operated on in a similar manner on September 1. Each lot was placed in a jar in three liters of sea water and both jars placed in the ice chest. One lot was fed and the other not fed. On September 14 nine tenths of those that had not been fed had survived and seven tenths of the others. There was no sign of regeneration in either lot. It might be added that in case of another experiment set up at the same time that regeneration was evident a few days after. (See Table III.)

It may be of interest to note that two of the survivors mentioned in the first experiment were placed in a shallow jar surrounded by running sea water, and in two weeks time the caudal fin showed a regeneration of 2 mm. indicating that just as soon as the temperature was raised to the normal regeneration began. It should also be noted that the temperature necessary to inhibit regeneration was not an extremely low one, and that this indicates that the power of regeneration is sensitive to lowering of temperature.

(B) Relation of Amount of Injury to Regeneration.

In 1902 Zeleny observed that when two chelæ of *Gelasimus*, the fiddler crab, were removed each of the regenerating buds grows more rapidly than does the single one when only one chela is removed. In 1903 the same author found that the regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the number of arms removed, *i. e.*, that animals with the greater number of arms removed regenerate more rapidly. The greater the degree of injury the more rapid the regeneration. In 1905 Zeleny in an experiment extending over 181 days using the common cray-fish, *Cambarus propinquus*, found that in the series with the greater degree of injury (Series B) each chela regenerates more rapidly than the single chela of those with the lesser degree of injury (Series A). in Series A consisting of 36 individuals the right chela was removed at its breaking joint. In series B consisting of 41 individuals the two chelæ and the last two pairs of walking legs were similarly removed. It is to be

regretted that the same author did not include a third series in which the injury was midway between that in A and B to see whether regeneration in that third series would be intermediate between that of series A and B. Thus Zeleny shows that in the cray-fish the greater degree of injury has a stimulating effect on regeneration.

On the other hand Emmel ('04) removed the right cheliped in the lobster immediately after moulting and allowed it to regenerate for the period between moults then removed it again after that moult and allowed it to regenerate. He found that it regenerated less between moults the more it was mutilated. In other words Emmel finds in a general way in a nearly allied form just the opposite result to Zeleny. In 1906 Emmel found a decrease in rate of moulting as correlated with greater degree of injury and lesser rate of regeneration, again a contradictory result to that of Zeleny. With a view of testing this question the following experiment was devised. Three lots of fishes were taken designated as *A*, *B*, *C*.

In *A* the caudal fin was amputated.

In *B* the caudal and one pectoral.

In *C* the caudal and both pectorals.

The fishes were kept in separate compartments in the hatchery with running sea water but were not fed. The experiment was begun on July 30 and continued until September 4 when all were removed and measured with fine pointed registering calipers. In the following Table I. are found the results of these measurements. Not only is the length of the specimen given but the amount of regeneration and the specific regeneration. This is a term used by Zeleny. Since the specimens differ in length we must devise some means of comparing regeneration in one case with that in another. We can do this by finding the percentage regeneration in each case, *i. e.*, by dividing the amount of regeneration by the length of the specimen. We may use Zeleny's term and call it specific regeneration. In the table is also given the weight of each specimen for purposes to be described later.

Now in interpreting these results we have recourse to the statistical method. We should apply certain formulæ here to ascertain whether differences in resulting regeneration in the three

different lots are great enough to ascribe any importance to them and thus say that they are due to difference in degree of injury—or on the other hand whether after all they are not simply chance discrepancies. Workers in statistical method have deduced the law that if the mean difference of one character in any two series of forms is less than the probable errors of the difference then the

TABLE I.

Lot A.

	Length Sp.	Amt Reg. Caudal.	Sp. Reg.	Wt. Sp.
1	8.1 cm.	.55 cm.	.0676	6.27 gm.
2	8.0	.65	.0813	5.80
3	6.5	.60	.0923	3.05
4	6.35	.80	.1259	3.38
5	7.25	.65	.0896	4.20
6	9.2	.65	.0706	11.55
7	10.2	.75	.0736	16.70
8	8.25	.80	.0969	8.4
9	9.4	.70	.0744	7.5
10	7.9	.60	.0760	6.25
11	6.35	.60	.0944	3.9
12	8.4	.80	.0952	10.12
13	8.4	.60	.0714	8.85

Lot B.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	11.3	.70	.0610	.55	.0486	26.87
2	7.1	.65	.0915	.50	.0704	4.75
3	8.3	.70	.0843	.60	.0723	7.60
4	8.2	.85	.1037	.60	.0732	10.52
5	7.7	.70	.0909	.60	.0779	5.42
6	11.1	.70	.0631	.65	.0585	22.47
7	8.8	.75	.0852	.60	.0682	9.80
8	7.6	.75	.0987	.60	.0789	11.75
9	9.15	.45	.0491	.45	.0492	10.32
10	6.4	.60	.0937	.50	.0781	2.85
11	5.4	.60	.1111	.50	.0926	2.11
12	6.9	.65	.0942	.60	.0869	4.60
13	5.5	.60	.1090	.30	.0545	2.05
14	8.6	.50	.0581	.50	.0581	5.95
15	6.15	.60	.0975	.50	.0813	3.15

Lot C.

	Length Sp.	Reg Ca.	Sp. Reg. Caudal.	Reg. r. Pec	Reg. l. Pec.	Ave. R. L Sp. Reg Pec	Wt. Sp.
1	11.5	.55	.0478	.55	.55	.0478	23.58
2	9.15	.85	.0929	.45	.45	.0492	12.37
3	9.7	.65	.0670	.50	.45	.0489	11.1
4	7.5	.60	.0800	.60	.60	.0800	5.72
5	7.5	.50	.0666	.35	.40	.0500	4.2
6	10.0	.75	.0750	.70	.70	.0700	14.6
7	6.3	.60	.0952	.50	.55	.0833	4.1
8	6.1	.76	.1065	.40	.50	.0737	3.08
9	5.8	.80	.1379	.45	.40	.0733	2.78

two series do not differ as to that character. If, however, the mean difference is 1 + times the probable difference then it is possible that the two series do differ as to the value of that character. Also if the mean difference is 2 + times the probable difference then it is probable that the two series differ. Finally if the mean difference is 3 + times the probable difference then it is certain that the two series differ as to the value of that character. To apply the law here it is necessary to find the mean of each series, the probable error, and from these compute the mean difference and the probable error of difference.

We can arrange the results as to the caudal fin in Table I. in the form of a table.

Mean Sp. Reg.	Probable Error.	Mean Difference.	Probable Difference.
Lot A = .0860	.003106	Between A and B .0009	Between A and B .004363
Lot B = .0851	.003065	Between B and C .0005	Between B and C .005865
Lot C = .0846	.004991	Between A and C .0014	Between A and C .005876

Comparing A and B we see at once that the mean difference is actually less than the probable difference, and hence this precludes the possibility of any rational conclusion that there is any difference in regeneration in cases A and B. But we have seen that injury in case of A was less than in B. Hence we cannot conclude that the rate of regeneration is greater in the case of the less injured nor in the case of the more injured. The regeneration is the *same*.

We find the same result when we compare B and C, and also when we compare A and C. This experiment tends therefore to negative the results of both Zeleny and Emmel.

We have thus far tested the question by comparing results in these three lots as to regeneration of the caudal fin. But we can also apply the test as to regeneration of the pectorals. We can average the results of the two pectorals in Lot C.

	Mean.	Prob. Error.	Mean Diff.	Prob. Diff.
Series B =	.0694	.002353		
Series C =	.0660	.002752	.0034	.0036

In this case also the mean difference is less than the probable

difference hence the two series are the same as to the amount of regeneration in the pectoral fin — although the amount of injury was different in the two cases.

TABLE II.

Lot D.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Wt. Sp.
1	9.9 cm.	.85 cm.	.0858	16.32 gm.
2	9.2	.70	.0760	13.15
3	8.6	.70	.0818	11.35
4	7.2	.80	.1111	11.52
5	8.9	.70	.0786	6.00
6	7.2	.80	.1111	4.09
7	6.4	.65	.1015	7.10
8	7.1	.85	.1197	6.80
9	7.6	.80	.1052	4.33
10	7.9	.75	.0949	7.22
11	6.1	.70	.1147	5.85
12	7.0	.75	.1071	8.30
13	4.5	.60	.1333	1.37

Lot E.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. R. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	8.6	.70	.0818	.60	.0697	11.00
2	10.35	.55	.0531	.60	.0579	16.52
3	7.5	.60	.0800	.40	.0500	6.00
4	7.8	.70	.0897	.60	.0897	8.25
5	8.5	.70	.0823	.50	.0588	11.35
6	7.8	.70	.0897	.55	.0705	8.6
7	8.1	.50	.0617	.50	.0617	9.00
8	10.3	.70	.0679	.70	.0679	17.87
9	8.8	.70	.0795	.50	.0568	10.06
10	6.2	.60	.0967	.40	.0646	4.6
11	8.7	.70	.0804	.50	.0574	13.52
12	6.4	.80	.1250	.50	.0781	5.32
13	8.4	.70	.0833	.60	.0714	10.72
14	6.0	.75	.1250	.50	.0833	3.95
15	7.4	.60	.0811	.50	.0675	8.20
16	6.2	.70	.1129	.55	.0887	4.15

Lot F.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. L. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	7.25	.60	.0827	.60	.0827	5.67
2	7.30	.60	.0821	.35	.0410	8.03
3	7.00	.70	.1000	.50	.0714	6.47
4	6.00	.70	.1166	.60	.1000	4.07
5	6.20	.70	.1129	.40	.0646	4.63
6	6.60	.70	.1060	.60	.1060	5.28
7	6.20	.60	.0967	.50	.0806	3.98
8	6.50	.65	.1000	.40	.0631	5.32
9	6.85	.70	.1022	.60	.0876	3.91
10	6.50	.60	.0923	.45	.0692	5.13
11	6.10	.70	.1147	.50	.0819	4.27
12	6.40	.75	.1172	.35	.1171	4.63

A second experiment was started on August 2 and continued until September 5 a period of four weeks and five days practically the same as those in Table I. In this case the fish were fed regularly for it was desired to test the effect of difference in food on regeneration. In Series D the caudal alone was cut, in E the caudal and right pectoral, in F the caudal and left pectoral. Following is the table of results, Table II.

We may arrange the results as before and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot D	.1010	.002956	Between D and E .0155	.004355
Lot E	.0855	.003199	Between D and F .0018	.003977
Lot F	.1028	.002699		

Since but one pectoral fin was cut off in both E and F it is clear that we cannot compare difference in regeneration in those two lots since the degree of injury was the same. In this case we compare E with D and F with D. Here is the first exception to the uniform results obtained in series A, B and C from Table I. For on comparing E with D we find that the mean difference in regeneration is more than three times the probable difference, hence according to our formula regeneration is really greater in D than in E. At first we might think that since the two differ as to degree of injury that this must be the cause of the result — that is, that in this case at least the least injured regenerated more in the same time. But is this the case? For comparing D and F (the injury to F being the same as that to E) we see that the mean difference between the two is less than the probable difference which shows that no importance can be assigned to that mean difference and that D and F regenerated practically the same amount in the same time. The case of D and F confirms the result already found in A–B, B–C and A–C as to caudal, and in case of B–C as to pectoral. The case of D–F also shows that whatever is the cause of the discrepancy between that and D–E we cannot say that E regenerated less because it was injured more than D.

TABLE III.

Lot G.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.
1	6.1	.30	.0491
2	6.2	.25	.0403
3	7.5	.15	.0200
4	6.9	.25	.0362
5	5.9	.30	.0508
6	6.5	.30	.0461
7	7.4	.20	.0270
8	8.1	.30	.0370
9	6.5	.20	.0307
10	7.0	.20	.0285
11	7.0	.30	.0428
12	7.0	.30	.0428
13	6.8	.20	.0293

Lot H.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.
1	9.0	.20	.0222	.20	.0222
2	7.2	.40	.0555	.20	.0277
3	6.5	.25	.0384	.25	.0384
4	7.7	.20	.0259	.20	.0259
5	6.2	.30	.0484	.25	.0403
6	6.4	.20	.0312	.20	.0312
7	6.8	.30	.0441	.30	.0441
8	7.1	.30	.0422	.25	.0352
9	7.2	.20	.0277	.10	.0138
10	6.4	.30	.0468	.20	.0312

Lot I.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.	Amt. Reg. Dors.
1	7.3	.30	.0410	.20	.0274	.10
2	6.6	.30	.0454	.25	.0378	.10
3	7.2	.30	.0416	.15	.0208	.10
4	6.5	.25	.0384	.30	.0461	.15
5	7.2	.20	.0277	.20	.0277	.15
6	7.5	.20	.0266	.15	.0200	.10
7	6.4	.30	.0468	.25	.0390	.10
8	7.1	.30	.0422	.25	.0352	.15

In order to test the same question further a third experiment was set up as follows: Three lots of fishes G, H and I were operated on as follows. In G the caudal fin alone was cut off; in H the caudal and one pectoral; in I the caudal, one pectoral and the dorsal. All the fish were placed in a large aquarium with running sea water and were fed regularly. The experiment was begun on September 1 and continued until September 14, 1906. Since all the fish were kept in the same tank there is opportunity for ascertaining whether the discrepancy in case of lot E might not be due to some condition peculiar to the com-

partment in which they were kept during the five weeks they were under observation. If the results in case of G–H and I confirm the results in cases A–B, A–C, B–C, D–F, etc., then I think we are warranted in concluding that some other condition other than degree of injury was responsible for that discrepancy in case of E. The fishes in this third experiment were fed every day or so. Following is Table III. giving the measurements of these fishes.

Treating these results as before we have the following table :

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot G	.0370	.001483	Between G and H .0005	.002611
Lot H	.0375	.002149	Between H and I .0013	.003539
Lot I	.0388	.002813	Between G and I .0018	.003179

We thus see that in this case also the mean difference is not great enough to ascribe any importance to it. In each case it is less than the probable difference and hence although the series differ as to degree of injury, they do not differ as to rate of regeneration. We can also tabulate the comparative regeneration in the case of the pectorals I and H.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot H	0.0312	.001871		
Lot I	0.0320	.001985	.0008	.002727

In this case also the mean difference is less than three times the probable difference which but corroborates the above contention. We therefore find that in nine cases the result is that although the fishes differed as to degree of injury, the rate of regeneration was the same. The experiments are certainly varied enough to represent different lengths of time, etc. There is but one exception to the results and that we have shown cannot be due to difference in degree of injury.

(C) Relation of Length and Weight (i. e., Age) of Specimen to Amount Rate of Regeneration.

Zeleny ('03) observed that the rate of regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the size of the animal — that the medium sized animals have the maximum rate of regeneration. We quote from his summary, "There is a definite relation between the size (age) of the animal and the rate of regeneration. The maximum rate is exhibited by individuals of medium size. Both the larger and the smaller ones give a diminishing rate as we go away from this point." Zeleny measures size and thus age by the disk width. The three tables given in this paper furnish us with data for determining whether regeneration in *Fundulus* is greater in the smaller, medium, or larger individuals. It occurred to the writer that we ought not only to take length but weight as an indication of age. Unfortunately

Table IV.

Shortest.			Medium.			Longest.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.
6.5	.60	.0923	8.1	.55	.0676	10.2	.75	.0735
6.35	.80	.1259	8	.65	.0813	11.3	.7	.0610
7.25	.65	.0896	9.2	.65	.0706	11.1	.7	.0631
6.35	.60	.0944	8.25	.8	.0969	11.5	.55	.0478
7	.65	.0915	9.4	.7	.0744	10.0	.75	.0750
6.4	.60	.0937	8.4	.8	.0952			
5.4	.60	.1111	8.4	.6	.0714			
6.9	.65	.0942	8.3	.7	.0843			
5.5	.60	.1090	8.2	.85	.1037			
6.15	.60	.0975	7.7	.7	.0909			
6.30	.60	.0952	8.8	.75	.0852			
6.10	.65	.1065	7.6	.75	.0987			
5.8	.80	.1375	9.15	.45	.0491			
7.5	.60	.0800	8.6	.5	.0581			
7.5	.50	.0666	9.15	.85	.0929			
			9.7	.65	.0670			
			7.9	6.0	.0760			

the specimens in Table III. were not weighed and hence will not be made use of in this comparison. It has been shown by others that regeneration is a phenomenon closely related to growth. Minot has established the fact that growth is greatest in the younger forms. Hence we should expect *a priori* that regeneration should also have a greater rate in the young than in the older forms. For the purposes of this study we can take the regene-

ration in the caudal fin of all the fishes represented in Table I. This irrespective of the fact that they differ as to degree of injury, since it has just been shown that that has not affected the rate of regeneration. In Table I, we have 37 specimens — the shortest is 5.4 cm. long while the longest is 11.5 cm. long. We can divide the 37 fishes into three lots (1) the shorter, those between 5.4 cm. and 7.5 cm.; the medium between 7.6 cm. and 9.7 cm. while the longer are between 9.8 cm. and 11.9 cm. Arranging all the specimens in these three groups we have the results shown in Table IV.

We find that the average specific regeneration of the shorter is .1028, that of the medium is .0797, while that of the longer is .0638. In other words, the shortest have regenerated 10 + per cent. of their own length, the medium-sized have regenerated 7 + per cent. of their length, while the longest have regenerated 6 + per cent. of their length. Thus the result is contrary to that found by Zeleny with the brittle star-fish. But to make certain we can employ the statistical method used above and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	.0972	.002823	Between S and M =.0190	.003698
Medium	.0782	.002390	Between M and L =.0096	.003786
Longest	.0686	.002934	Between S and L =.0286	.004071

Now we find that the mean difference between S and M is not only more than three times the probable difference but nearly six times as large so that there can be no question but that the shortest have regenerated at a greater rate. On comparing S with L we find that the mean difference in regeneration is over seven times the probable difference. So that our conclusion is that the shorter have regenerated at a much greater rate than either the medium or the longer and that also the medium have regenerated at a greater rate than the longest. Now this last is confirmed when we compare M and L. In this case the mean difference is not quite three times the probable difference but more than twice the probable difference so that it is more than probable that the medium have regenerated more than the longer.

But we also have Table II. for comparison. The shorter specimens in this series are those between 4.5 cm. and 6.45 cm; the medium between 6.46 and 8.41 and the longer between 8.42 and 10.37 cm. Arranging the specimens we have Table V.

TABLE V.

Shorter.			Medium.			Longer.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.
6.4	.65	.1015	7.2	.8	.1111	9.9	.85	.0858
6.1	.7	.1147	7.2	.8	.1111	9.2	.7	.0760
4.5	.6	.1333	7.1	.85	.1197	8.6	.7	.0818
6.2	.6	.0967	7.6	.8	.1052	8.9	.7	.0786
6.4	.88	.1250	7.9	.75	.0949	8.6	.7	.0818
6	.75	.1250	7	.75	.1071	10.35	.55	.0531
6.2	.7	.1129	7.5	.6	.0800	8.5	.7	.0823
6	.7	.1166	7.8	.7	.0897	10.3	.7	.0679
6.2	.7	.1129	7.8	.7	.0897	8.8	.7	.0795
6.2	.6	.0967	8.1	.5	.0617	8.7	.7	.0804
6.1	.7	.1147	8.4	.7	.0843			
6.4	.75	.1172	7.4	.6	.0811			
			7.25	.6	.0827			
			7.3	.6	.0821			
			7	.7	.1000			
			6.6	.7	.1060			
			6.5	.65	.1000			
			6.8	.7	.1022			
			6.5	.6	.0923			

Treating the results as before, we find the average specific regeneration for the shortest is .1136, that of the medium is .0944 while that of the longest is .0763. In other words the shortest of this series of fed fishes have regenerated 11+ per cent. of their length, the medium sized have regenerated 9 + per cent. of their length while the longest have regenerated 7 + per cent. of their length.

In this case also we can apply the statistical method tabulating our results in the following form.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	=.1137	.002897	Between S and M =.0203	.003492
Medium	=.0934	.001949	Between M and L =.0170	.003066
Longest	=.0764	.002367	Between S and L =.0373	.003741

Here again it will be seen that the mean difference in regeneration between S and M is over three times the probable difference, in fact nearly six times; the mean difference between S and L shows that it is over nine times the probable difference so that there can be no doubt that the shortest specimens in Table II. regenerated at a more rapid rate than did the medium or the longest. On comparing the medium with the longest we find that the medium regenerated more rapidly than the longest as is evidenced by the fact that the mean difference is over five times the probable difference.

If we extend our hypothesis that there is a direct relation between growth and regeneration further it would be to find out whether as the fish grew older, the rate of regeneration decreased. For this is precisely the condition as regards growth — the rate of growth decreases with age. At first the decrease is rapid but then becomes very gradual. This is indicated with regard to regeneration. For on comparing regeneration of the shortest with the medium and the regeneration in the medium with the longest we discover an indication of this relation. In each of the two cases it is seen that mean difference in regeneration is greater between shortest and medium as compared with the mean difference in regeneration between the medium and longest.

It was said at the beginning of this section that in estimating age we ought to consider both length and weight. So I have placed opposite the length, etc., of each specimen its weight. *A priori*, the youngest ought to be the shortest and lightest, etc. This is the case here, for the average weight of the shortest is 3.38 gm.; the average weight of the medium length is 8.96 gm., while that of the longest is 20.85 gm. So that in Table IV. our three classes represent the youngest, the oldest and those midway between the others in age. In the same way for Table V. the average weight of the shortest is 4.48 gm.; that of the longest is 12.71 gm. while that of the medium length is 7.52 gm. In this case also our three series in Table V. represent the youngest, the oldest and the medium aged fishes of that lot. We conclude therefore that the rate of regeneration in *Fundulus h.* is greatest in the youngest fishes, less in the older, and that there is a slight indication that the rate of regeneration decreases with age.

(D) Relation of Regeneration to Food.

It will be remembered that fishes in Series A, B and C were not fed while those in Series D, E and F were fed. Otherwise as far as known they were kept under identical conditions and for the same length of time. It was desired to compare regeneration in the two series with each other to ascertain whether regeneration is at a greater rate in the fed fishes than in the not-fed. Unfortunately we cannot make use of Series E because as before said some factor has entered there and caused a result which I do not believe to be normal. On the other hand I do regard Series D and F as normal. Therefore we must compare regeneration in D with that in A.

I. Caudal.	Mean Reg.	Prob. Error.	Mean Diff	Prob. Diff.
Lot D (fed)	=.1010	.003106		
Lot A (not fed)	=.0860	.002958	.0150	.004249

In this case the mean difference in regeneration is more than three times the probable difference. In other words the fishes in Lot D which were fed have regenerated more in the same time than the fishes in Lot A which were not fed.

II. Caudal.	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot F (fed)	=.1028	.002659		
Lot B (not fed)	=.0851	.003065	.0177	.004084

In this case also the mean difference in regeneration between F and B is more than three times the probable difference. Or the fishes in Lot F which were fed have regenerated more than the fishes in Lot B which were not fed.

We may compare the average specific regeneration of the caudal in the case of the fishes that were fed with that in the fishes that were not fed in the following way also.

Average Sp. Reg. in caudal of Lots D + F (fed)..... =0.10100
Average Sp. Reg. in caudal of Lots A + B + C (not fed)..... =0.08565
Difference..... =0.01535

The caudal fin in fishes that were fed regenerated 18 per cent. more than that in fishes not fed.

Again,

Average Sp. Reg. in pectoral fin in Lot F (fed)..... =0.0866
Average Sp. Reg. in pectoral fin in Lot B + C (not fed)..... =0.0677
Difference..... =0.0189

that is, the pectoral in the fed fishes regenerated 24 per cent. more than that in the unfed.

(E) Regeneration in Fins used most Compared with that in Less used Fins.

Adaptation in Regeneration. — Osburn ('06) experimenting on the fins in *Fundulus* finds that the pectorals are not used as vigorously as the caudal, and the dorsal not so much as the pectorals. Broussonet (1786) stated that the most useful fins regenerate more rapidly than those less useful. Morrill ('06) says that the "caudal fin though obviously the most important does not regenerate perceptibly faster than the others." Although the data are insufficient to settle this question which is to a great extent a matter of interpretation yet the following table is suggestive. Since it has been shown that degree of injury has made no difference in the rate of regeneration in *Fundulus h.*, it is therefore possible to group all the specimens represented in Table I. thus,

Average Sp. Reg. of caudal fin in Lots A + B + C	= 0.08565
Average Sp. Reg. of pectoral fin in Lots B + C	= 0.06770
Difference	= 0.01795

which means that the caudal fin of specimens in Lots A + B + C regenerated 26 per cent. more than the pectoral fin in Lots B + C in the same time and under same conditions.

In the same way from Table II., we find

Average Sp. Reg. of caudal fin in Lots D + F	= 0.01016
Average Sp. Reg. of pectoral fin in Lot F	= 0.00846
Difference	= 0.00170

or in other words the caudal regenerated 20 per cent. more than the pectoral.

Again in Table III. the

Average Sp. Reg. in caudal in Lots G + H + I	= 0.0386
Average Sp. Reg. in pectoral in Lots H + I	= 0.0313
Difference	= 0.0073

or the caudal in this case has regenerated 20 per cent. more than the pectoral. Thus in all three cases the result is the same. It should be remembered that specific regeneration is the relation of amount regenerated to the length of the specimen. And hence we have a right to compare the specific regenerations of caudal with pectoral. It may also be pointed out here that the dorsal

fin regenerates very slowly as compared with the other two as may be seen in Table III.

General Conclusions.

1. That low temperatures inhibit regeneration in caudal fin of *Fundulus heteroclitus.*

2. That the rate of regeneration bears no relation to the degree of injury to the caudal and pectoral fins.

3. That regeneration is greater in the younger than in the medium and older fishes which is in line with the theory that regeneration is a growth phenomenon.

4. That regeneration is greater in the fish that have been fed as compared with that in the fishes not fed.

5. That there *is* an indication that the fins used or needed most (*i. e.*, caudal) regenerate more rapidly than the less needed fin (pectoral), or in other words there is an indication of adaptation in regeneration.

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